Factors affecting the voluntary intake of food by sheep. 5. The inhibitory effect of hypertonicity in the rumen

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The site where osmotically active substances act to depress food intake was determined in sheep. After 5·5 h of food deprivation, solutions of sodium chloride or polyethylene glycol-200 (PEG-200) were added to either the reticulo-rumen or the abomasum. The sheep were then immediately offered pelleted lucerne (*Medicago sativa*). Water was withheld during the first 60 min of feeding but was available from 60 to 90 min. There was a linear inhibition in food intake in the first 10 min after loading 2·37, 6·25, 12·5, 25·0 or 50·0 g NaCl into the rumen according to a 5×5 Latin square design ($P = 0·0001$). The intake reduction was 3·49 g food/g NaCl. An osmotic load of PEG-200 equivalent to 50 g NaCl also significantly inhibited food intake in the first 10 min of the meal compared with a control treatment. The inhibition of food intake after loading 55 g NaCl into the rumen was not affected by injecting lidocaine hydrochloride into the reticulum immediately before NaCl loading. NaCl injected into the abomasum did not significantly affect food intake in the first 10 min of feeding even though the tonicity of abomasal digesta was increased to unphysiological levels. There was no consistent relationship between food intake and the change in the tonicity of jugular plasma following solute loading and drinking. The sensing site of hypertonicity was localized to the wall of the reticulo-rumen where neuronal receptors appear to be capable of detecting osmotic pressure within the physiological range to depress food intake. These receptors should be identified and characterized because of their possible significance in limiting food intake by ruminants.

Osmolality: Food intake: Sheep

Short-chain fatty acid (VFA) production and the elution of minerals from ingested food result in an increase in the osmolality of rumen fluid, the rate and extent of which depends on the nature and amount of the food consumed. An increase in plasma osmolality arises from the absorption of electrolytes and VFA and from the secretion of fluid into the gut. The latter response increases packed cell volume and plasma protein concentration.

A linear reduction in the intake of lucerne (*Medicago sativa*) hay was observed after hypertonic solutions of sodium chloride, potassium chloride and the sodium salts of acetic, propionic and butyric acids were added to the rumen immediately before feeding (Ternouth & Beattie, 1971). The reduction in food intake was greater in the first hour of the 2 h feeding period each day. These authors also showed that intake was significantly increased when water was added to the rumen up to 1 h before feeding. Similarly, Bergen (1972) added either sodium acetate or NaCl to the rumen of sheep and inhibited daily intakes consumed over a 2 h period. The infusion of hypertonic extracts of fresh and ensiled whole-plant maize and NaCl solutions into the rumen of sheep commencing 3 h before lucerne pellets were offered led to a linear decrease in food intake during the first 30 min of eating, and was associated with a linear increase in osmolality of rumen fluid (Phillip *et al.* 1981). The mechanism accounting for the observations cited in the three papers mentioned previously is unclear, because after 1 or 2 h of eating or after infusing solutions for 3 h before feeding,

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sufficient time would have elapsed for osmolality changes to occur in blood and abomasal digesta as well as in the rumen. Bergen (1972) found that 20 mg carbocaine added to the rumen along with either 90 g sodium acetate or 60 g NaCl blocked the intake depression in the subsequent 2 h feeding period. However, the site of action of carbocaine 2 h after rumen infusion is unknown. The mechanism of action of the carbocaine, therefore, remains unclear.

Experiments were designed to ascertain if the intake depression from NaCl loading of the rumen was mediated by the reticulo-rumen, abomasum or via the circulation. The results demonstrate that the intake inhibition due to osmotically loading the rumen was caused by the increase in osmolality of rumen digesta and not by increases in the tonicity of abomasal digesta or blood.

EXPERIMENTAL

Sheep and surgery

Crossbred Suffolk wethers were used in all experiments. Cannulas were inserted in the rumen (Grovum, 1988a) and abomasum (Hecker, 1974) of each sheep in one operation leaving 14 d for recovery before commencing preliminary experiments. The sheep (n 6) weighed 46·2 (SE 2·5) kg at surgery (approximately 6 months of age) and were 82·6 (SE 5·4) kg at the completion of experiments (12 months of age). At post mortem the sheep had considerable subcutaneous and intra-abdominal fat. However, the latter should not have affected food intake as the diet was ground and pelleted and so had a high bulk density, thus limiting the degree of reticulo-ruminal distension (Grovum, 1988b). The sheep were treated for internal and external parasites with ivermectin before the start of experiments (Ivomec; MSDAGVET, Division of Merck Frosst Canada Inc).

Housing and food

The sheep were held in metabolism crates in a temperature-controlled room (22–24°) with 24 h lighting. Fresh water was available at all times other than during experiments as described under protocol. Ground and pelleted lucerne (Medicago sativa) hay was available ad lib. except for short periods of deprivation on experimental days and contained (g/kg): 908 dry matter, 165 crude protein (nitrogen × 6·25), 44 fat, 79 ash, 323 acid-detergent fibre, 14 calcium, 3 phosphorus, except in Expts 4 and 5 when it contained 927 dry matter, 171 crude protein, 14 fat, 72 ash, 370 acid-detergent fibre, 15 calcium, 3 phosphorus.

General experimental protocol

The sheep were fed on lucerne pellets ad lib. but on the day of an experiment, fresh food and water were provided from 08.00 to 09.00 hours and then food was withheld for 5·5 h to induce a moderate level of hunger against which the apparent satiating effects of the treatments were assessed. Water was withdrawn at 14.00 hours. NaCl was dissolved in distilled water and made up to the solution volume stated. All solutions were warmed to body temperature before being added to either the reticulo-rumen or the abomasum immediately before feeding at 14.30 hours. Food intake was measured after 10, 20, 30 and 60 min. At 60 min, water was provided as well as food, and both water and food intake were measured for the next 30 min. Food and water were then available until 08.00 hours the following morning at which time both intakes were again recorded. Treatments were imposed on successive days because preliminary experiments in which water consumption and urine output were measured showed that 50 g NaCl loaded into the rumen was cleared from the sheep well within 15 h. Food intake was only affected for up to 4 h after NaCl loading. Preliminary experiments also showed that neither the frequency nor the amplitude of reticular contractions recorded manometrically as described by Grovum (1981), were affected by the NaCl loading.

Digesta were obtained from the rumen using a sampling probe positioned in the ventral sac or in the case of the abomasum, by collecting outflow from the abomasal cannula. The rumen probe consisted of a 350–400 mm length of stiff polyethylene tubing (5 mm i.d., 7 mm o.d.) through which a narrower polyethylene tube (2 mm i.d., 3 mm o.d.) was fed until it protruded about 2 mm. The stiff, outer tube afforded rigidity to the inner tube through which digesta were sampled. The gap between the two tubes at the sampling end was sealed with a 7 mm length of rubber tubing. A 3 cm² piece of mesh (Proxplast, eight holes/cm, Goshen Labs, 36-T, St John's Street, Goshen, N.Y., USA) was firmly tied over the tip. A piece of nylon stocking was then secured over the mesh. These materials acted as filters and prevented particulate matter from obstructing the inner tube during sampling. The other end of the probe passed through a rubber stopper in the rumen cannula and was fitted with a three-way stopcock for sampling. About 10 ml air were injected before sampling to clear the sampling tube, and digesta were withdrawn and re-injected twice before the third volume (5–15 ml) of digesta were taken as the sample.

Blood was obtained by jugular venipuncture as blood could not be drawn reliably from jugular catheters after 2 d. Blood samples (5 ml) were immediately placed on ice in heparinized tubes and centrifuged within 2 h to obtain plasma. Both plasma and digesta samples were frozen ($-20°$) until being analysed for osmolality by freezing point depression with a digimatic osmometer (model 3DII; Advanced Instruments Inc, Needham Heights, Mass., USA).

Intake is a highly variable measure. It was, therefore, necessary to first conduct experiments to collect intake values only, without interference to the animal. When digesta and blood sampling were necessary, the entire experiment or selected treatments were repeated exactly as before and samples were taken accordingly.

Expt 1. NaCl loading of the reticulo-rumen

(a) NaCl loads (2·37, 6·25, 12·5, 25 and 50 g) in 300-ml solutions were drained into the dorsal sac of five sheep according to a 5 × 5 Latin square design. The 2·37 g treatment solution was the control as its tonicity was 270 mosmol/kg. The left side of each sheep was briefly palpated to facilitate mixing of the solution with rumen contents. A period of 2 min was then allowed before food was offered.

(b) In a crossover-type design, the control and 50 g NaCl treatments were reimposed on successive days in five sheep to collect digesta and blood samples. Rumen digesta and blood were taken just before dosing the rumen and after 10, 60 and 90 min of feeding.

Expt 2. Polyethylene glycol (PEG-200) loading of the reticulo-rumen

The objective of the present experiment was to determine the effect of an indigestible organic osmotic load on food intake. Experiment 1(b) was repeated except that PEG-200 (molecular weight 200) was substituted for NaCl to achieve identical osmotic loads calculated on the basis of molecular weight. The high load (342·2 g) occupied 310 ml and so the control load of 16·2 g was also made up to this volume with distilled water. Rumen digesta and blood samples were collected as described in Expt 1(b), however, tonicity changes of rumen fluid could not be determined when it contained PEG-200. This is due to the unique reaction of PEG with water causing an anomalous depression in freezing point (Blow et al. 1978; Schiller et al. 1988). It was assumed that equal osmotic loads of NaCl and PEG would have equal osmotic effects in the reticulo-rumen.

Expt 3. Lidocaine and NaCl loading of the reticulo-rumen

The aim of the present experiment was to determine whether a local anaesthetic in the rumen fluid could block the intake depression caused by NaCl loading. Lidocaine was selected as it is a potent local anaesthetic, is readily soluble in water and is well absorbed

through the epithelium of the gastrointestinal tract (Goodman & Gilman, 1985). Unfortunately the extent of lidocaine metabolism by rumen bacteria is unknown. In a preliminary experiment on one sheep, 4 g lidocaine hydrochloride (xylocaine (20 g/l); Astra Pharmaceuticals Canada Ltd, Mississauga, Ontario), directed entirely toward the reticulum via a stiff polyethylene tube passed through the rumen cannula, had no effect on reticular motility. However, 8 g inhibited the frequency of reticular contractions by 39 % with no other apparent untoward effects on the animal.

Before feeding at 14.30 hours, six sheep received two injections into the reticulo-rumen by syringe connected to a stiff polyethylene tube. The first injection containing anaesthetic (400 ml) was directed entirely toward the reticulum. The second injection containing NaCl (200 ml) was directed so that approximately equal volumes were distributed into the reticulum, dorsal sac and dorsal blind sacs. The six treatments were as follows: (1) 400 ml water followed by 1·54 g NaCl (control NaCl), (2) 4 g lidocaine followed by 1·54 g NaCl (low lidocaine control), (3) 8 g lidocaine followed by 1·54 g NaCl (high lidocaine control), (4) 400 ml water followed by 55 g NaCl, (5) 4 g lidocaine followed by 55 g NaCl, (6) 8 g lidocaine followed by 55 g NaCl.

The 400 ml volume of the first injection was established by the concentration of the lidocaine hydrochloride solution (20 g/l) giving the high dose of 8 g. Treatments were imposed according to a 6 × 6 Latin square which was balanced for residual effects (Cochran & Cox, 1957).

Expt 4. NaCl loading of the abomasum

(a) The objective of this experiment was to determine whether the abomasum was a site where tonicity may be sensed to inhibit food intake. NaCl (0·41, 0·89, 1·79, 3·57 and 7·14 g) in 50-ml solutions was injected into the abomasum of each of five sheep immediately before offering food at 14.30 hours. The control dose of 0·41 g NaCl was calculated to maintain tonicity of abomasal digesta at about 282 mosmol/kg which was an assumed 'control' value (von Engelhardt & Hauffe, 1975). The other NaCl amounts were calculated by scaling down the loads used in Expt 1 (a) assuming abomasal and rumen volumes of 0·5 and 3·5 litres respectively. A rumen fluid volume of 3·5 litres was thought to be reasonable considering that the diet was ground and pelleted (Faichney & Boston, 1985).

(b) At the completion of this experiment the 7·14 g NaCl treatment was imposed as described previously in the five sheep but with abomasal digesta samples taken to assess tonicity changes. Control abomasal digesta samples were taken 30 min before injection. Digesta samples were then taken 5, 10 and 15 min after food was offered.

(c) The effect of a further increased NaCl load into the abomasum (14·3 g) on food intake was determined in a cross-over-type experiment with five sheep. Solutions (50 ml) containing either 0·41 g or 14·3 g NaCl were injected into the abomasum and intake was measured as described previously.

Expt 5. PEG-200 and NaCl loading of the abomasum

The aims of this experiment were to determine if food intake could be inhibited in the first 10 min of eating with a high NaCl load into the abomasum, and to test an iso-osmotic load of an alternative organic solute. The treatments were 1·65 (control), 14·3 and 28·6 g NaCl, 98 and 196 g PEG-200 in 200 ml solutions. The low and high loads of NaCl and PEG-200 were equivalent in osmotic strength. The volume injected was 200 ml to accommodate 196 g PEG. The treatments were imposed according to a 5 × 5 Latin square design.

On completion of this experiment, the three NaCl treatments were reimposed on all five sheep in two cross-over-type design experiments to sample digesta and blood. Each sheep received each treatment in a random sequential order over a 3 d period with blood sampling

conducted in the first experiment and abomasal digesta sampling in the second experiment. Abomasal digesta samples were taken 17 min before injection and feeding and 9, 14, 24 and 35 min later. Blood samples were taken just before injection and after 10, 60 and 90 min of eating. Digesta and blood were sampled separately to keep the time-period spent collecting the samples to a minimum and, thus, more closely represent the time course of changes that would have occurred in the intake experiment.

Expt 6. NaCl loading of the reticulo-rumen and abomasum

(a) This 6×6 Latin square experiment was designed to test both the reticulo-rumen and abomasal responses to NaCl loading within the same experiment. The rumen treatments included 1·34 (control), 25 and 50 g NaCl in 175-ml solutions which were injected so that approximately equal volumes were distributed to the reticulum, dorsal sac and dorsal blind sacs. Brief palpation of the left side of the animal followed the injections. The abomasal treatments included 0·41 (control), 7·14 and 14·3 g NaCl in 50 ml solutions. Brief palpation in the region of the abomasum followed the injection. Food was offered immediately after all sheep were injected and palpated.

(b) The treatments in Expt 6(a) were reimposed so that rumen and blood samples could be collected. Rumen sampling probes were inserted in the morning with blood and digesta samples collected just before injecting the NaCl solutions and after 10, 20, 30, 60 and 90 min of eating.

Statistical analysis

Regression analysis relating intake to tonicity changes in digesta and blood, as well as analysis of variance of the intake values using the general linear models procedure was used. The Newman–Keuls test was used for comparing means. In Expt 1 a partial R^2 was used to describe the relationship between food intake and the NaCl load into the rumen. The partial R^2 as defined by Ormrod et al. (1986) is RSS/(RSS + ESS), where RSS in Expt 1 is the regression (model) sum of squares after subtracting the (unadjusted) sum of squares due to sheep and period, and ESS is the error sum of squares.

RESULTS

Expt 1. NaCl loading of the reticulo-rumen

The addition of NaCl solutions to the rumen immediately before offering lucerne pellets in Expt 1(a) resulted in a significant linear depression in intake during the first 10 min of eating ($P = 0·0001$; Fig. 1). After removing the effects of sheep ($P = 0·01$) and period (not significant), food intake during this first 10 min period can be explained by equation 1:

$$y = 324 - 3·49x, \tag{1}$$

where y is intake (g) and x is NaCl load into the rumen (g). This dose-response relationship indicates that the addition of 1 g NaCl to the rumen would inhibit intake by 3·49 g within a 10 min period. After removing sheep and period effects, the 'partial R^2' for the response was 0·68. The linear pattern of intake depression was maintained in cumulative intakes up to 60 min. The extent of the intake depression, as indicated by the slope value, increased to 5·34 g/g NaCl after 30 min (slope $-5·34$). The NaCl loads resulted in a linear increase in both water consumption ($P = 0·0001$) and food intake ($P = 0·02$) from 60 to 90 min.

Adding 50 g NaCl to the rumen increased the rumen osmolality in 10 min by 249 (SE 79) mosmol/kg compared with 33 (SE 6) mosmol/kg for the controls (Fig. 2). The corresponding values for changes in jugular plasma osmolality were $+5·8$ (SE 1·1) and $-0·1$ (SE 1·0) mosmol/kg respectively. Hence, the depression in food intake with 50 g NaCl in the rumen was associated with an increase in both rumen and plasma tonicity. When the
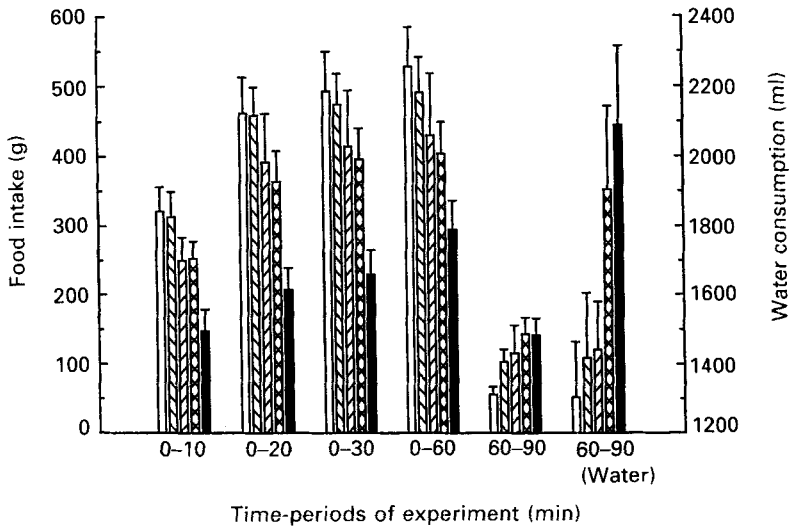
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Fig. 1. Expt 1 (a). Effect on food intake and water consumption of injecting 2·37 (control; ☐), 6·25 (◳), 12·5 (▨), 25·0 (⊠) or 50·0 (■) g sodium chloride into the rumen of sheep that were deprived of food for 5·5 h (n 5). NaCl was injected immediately before feeding. Intake is displayed for the time periods 0–10, 0–20, 0–30, 0–60 min when water was withheld and from 60 to 90 min when water was available. Values are means with their standard errors represented by vertical bars.
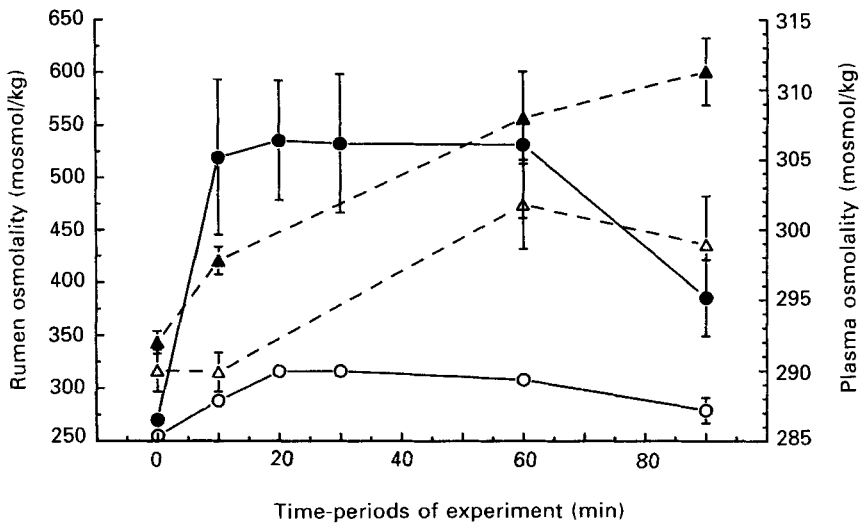

Fig. 2. Expt 1 (b). Effect on osmolality of rumen fluid and plasma of injecting 2·37 (control) or 50 g sodium chloride into the rumen immediately before feeding sheep (time 0) after a 5·5 h deprivation period. Water was withheld from 0 to 60 min but was available from 60 to 90 min. (——), Osmolality of rumen fluid; (– – – –), osmolality of plasma (n 5). (○), Control rumen osmolality; (●), 50 g NaCl rumen osmolality; (△), control plasma osmolality; (▲), 50 g NaCl plasma osmolality. Values are means with their standard errors represented by vertical bars.

individual sheep intakes for the first 10 min from Expt 1 (a) were regressed v. the changes in rumen or plasma tonicity from Expt 1 (b), the model using rumen osmolality approached significance ($P = 0.053$; R^2 0·39), but that using plasma osmolality was not significant ($P > 0.1$; R^2 0·17). Equation 2 relating intake to the change in rumen tonicity is:

$$y = 296 \cdot 3 - 0 \cdot 44x, \tag{2}$$

where y is food intake (g) and x is the change in tonicity of rumen fluid in mosmol/kg from 0 to 10 min. Water consumption from 60 to 90 min decreased rumen osmolality by 146 (SE 53) mosmol/kg on the 50 g NaCl treatment compared with 29 (SE 8) mosmol/kg for the controls. However, jugular plasma osmolality increased by 3·3 (SE 1·3) mosmol/kg for the 50 g NaCl treatment, whereas it decreased by 2·9 (SE 2·2) mosmol/kg for the controls. At 60 min, the plasma osmolality was also higher (308 (SE 3·3) mosmol/kg) for the 50 g NaCl treatment than for the controls (301·8 (SE 3·2) mosmol/kg). Thus, despite the already considerably elevated plasma osmolality for the 50 g NaCl treatment and a further increase of 3·3 mosmol/kg from 60 to 90 min, NaCl-treated sheep in Expt 1 (a) consumed 2·54 times more food than the controls. Intake from 60 to 90 min was not related to the change in plasma osmolality ($P > 0·1$), but the model relating intake to the change in rumen osmolality was highly significant ($P = 0·0001$; R^2 0·87) as described by equation 3:

$$y = 50·1 - 0·56x, \tag{3}$$

where y is intake (g) and x is the change in tonicity of rumen fluid in mosmol/kg from 60–90 min. The negative relationships relating intake and tonicity of rumen digesta indicate that raising rumen fluid tonicity by NaCl loading will depress intake, whereas reducing rumen tonicity by drinking water will increase intake.

Expt 2. PEG-200 loading of the reticulo-rumen

The food intake patterns observed with PEG-200 at osmotic loads equivalent to the control and 50 g NaCl treatments used in Expt 1, were similar to those reported previously. The intake depression observed in the first 10 min was significant ($P = 0·02$) and water consumption from 60 to 90 min was greater ($P = 0·002$) with the high-PEG treatment (2092 (SE 265) ml) compared with the control (1264 (SE 34) ml). The 60 to 90 min food intake on the high-PEG treatment (143 (SE 34) g) was also greater than controls (47 (SE 21) g; $P = 0·01$; Fig. 3).

It was assumed that absorption of PEG into blood would have been negligible over the 90 min period after rumen loading. The changes in plasma osmolality were similar to that observed in Expt 1. After 10 min, plasma osmolality rose by 4·4 (SE 0·5) and 0·7 (SE 0·9) mosmol/kg for the high-PEG and control treatments respectively. The tonicity increase due to PEG was attributed to a movement of water from blood into the hypertonic rumen contents. Intake in the first 10 min was not related to the change in plasma osmolality. From 60 to 90 min, plasma osmolality increased 1·9 (SE 1·4) mosmol/kg on the high-PEG treatment compared with a decrease of 2·2 (SE 1·0) mosmol/kg on the control treatment. Plasma osmolality at 60 min was 10·6 mosmol/kg higher on the high-PEG treatment (306·2 (SE 1·4) mosmol/kg) than controls (295·6 (SE 1·8) mosmol/kg). Thus, despite an already considerably elevated plasma osmolality and a further rise of 1·9 mosmol/kg from 60 to 90 min, there was a significant increase in food intake on the high-PEG treated sheep compared with the control treatment once the animals were permitted to drink. Intake from 60 to 90 min was related to the change in plasma osmolality ($P = 0·01$), but it had a positive slope.

Expt 3. Lidocaine and NaCl loading of the reticulo-rumen

Intakes on the three 55 g NaCl treatments (plus 0, 4 or 8 g lidocaine) were significantly less than those for the controls (1·54 g NaCl alone or with 4 or 8 g lidocaine) in the first 10 min after dosing the rumen. The mean intakes for the three 55 g NaCl treatments and the three control treatments were 54·3 (SE 7) g and 310·4 (SE 20) g respectively (Fig. 4). There were no differences between the three control treatments (i.e. no effect of lidocaine alone) or between the three high-NaCl treatments with and without lidocaine. Hence, lidocaine did not block the inhibitory effect of rumen NaCl loading on intake.
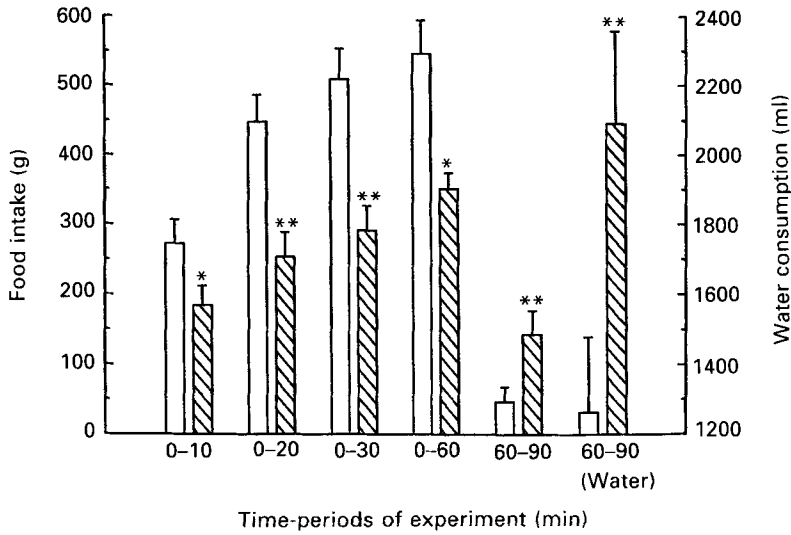
Fig. 3. Expt 2. Effect on food intake and water consumption of injecting 16·2 (control; □) or 342·2 (▨) g polyethylene glycol (molecular weight 200; PEG) into the rumen of sheep that were deprived of food for 5·5 h (*n* 5). PEG was injected immediately before feeding. Intake is displayed for the time periods 0–10, 0–20, 0–30, 0–60 min when water was withheld and from 60 to 90 min when water was available. Values are means with their standard errors represented by vertical bars. The difference between treatment means was significantly different: *$P < 0.05$, **$P < 0.01$.

Whilst there were no significant treatment effects on intake from 60 to 90 min, the same pattern was observed as seen in Expts 1 and 2 for the high-NaCl load and control treatments. The 55 g NaCl (no lidocaine) treatment resulted in a mean intake of 198 (SE 39) g compared with 87 (SE 30) g for the controls. There was, however, a significant effect of treatment on water consumption for this period ($P = 0.002$). Sheep on the 55 g NaCl (no lidocaine) treatment consumed significantly more water than those receiving the control NaCl treatments and the 55 g NaCl plus 8 g lidocaine, but there was no difference in water consumption between the 55 g NaCl plus 4 and 8 g lidocaine treatments and controls.

Expt 4. NaCl loading of the abomasum

Increasing the NaCl load into the abomasum from 0·41 g through to 7·14 g in Expt 4(a) did not affect food or water intake in any time-period (e.g. 0–10 min intakes for 0·41, 0·89, 1·79, 3·57 and 7·14 g NaCl were 219 (SE 44), 267 (SE 22), 226 (SE 17), 255 (SE 24) and 262 (SE 20) g respectively). However, there was a significant linear relationship between NaCl injected and water consumption from 60 to 90 min ($P < 0.05$). This drinking response did not influence intake for this period as was seen in the rumen studies. Abomasal tonicity increased from a predosing mean of 286 (SE 7) to 583 (SE 41) mosmol/kg 5 min after 7·14 g NaCl were injected into the abomasum. After 10 and 15 min, the tonicity values were 439 (SE 12) and 375 (SE 16) mosmol/kg respectively.

Increasing the amount of NaCl injected into the abomasum to 14·3 g in Expt 4(c) still did not affect food intake in the first 10 or 30 min compared with the controls. However, the intake difference from 10 to 20 min was significant (135 (SE 16) and 185 (SE 28) g for 14·3 g NaCl and control treatments respectively; $P = 0.05$). There was a significant difference in water consumption from 60 to 90 min ($P = 0.02$) with 2399 (SE 380) and 1564 (SE 199) ml being consumed for 14·3 g NaCl and control treatments respectively.
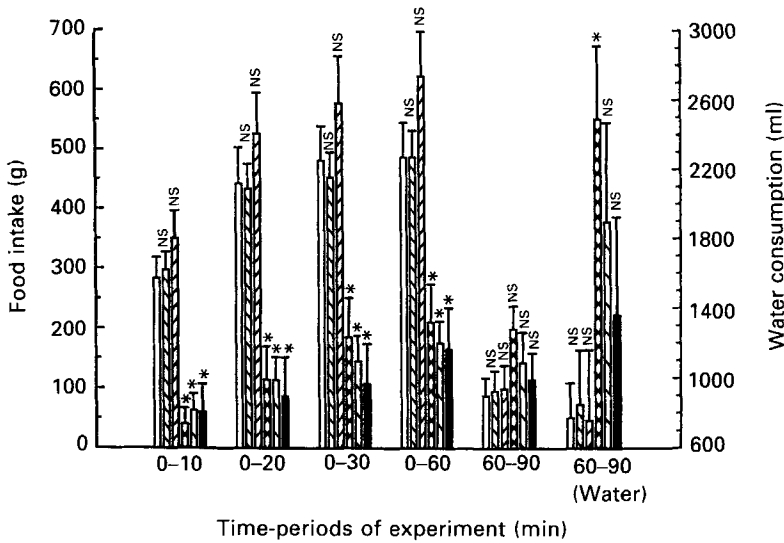
Fig. 4. Expt 3. Effect on food intake and water consumption of injecting a local anaesthetic into the reticulum with and without sodium chloride in sheep that were deprived of food for 5·5 h. The control treatment was 1·54 g NaCl (□). The 4 (◨) or 8 (▨) g lidocaine hydrochloride treatments included 1·54 g NaCl. The high-NaCl load (55 g) was injected either alone (⊠) or with 4 (◩) or 8 (■) g lidocaine (n 6). The lidocaine and NaCl were injected into the reticulum and reticulo-rumen respectively immediately before feeding. Intake is displayed for the time periods 0–10, 0–20, 0–30, 0–60 min when water was withheld and from 60 to 90 min when water was available. Values are means with their standard errors represented by vertical bars. Treatment means were significantly different from those for controls for each time period using the Newman–Keuls test: *P < 0·05, NS, not significant.

Expt 5. PEG-200 and NaCl loading of the abomasum

Loading the abomasum with 14·3 g NaCl and the equivalent osmotic load as PEG-200 did not depress food intake from 0 to 10 min (Fig. 5). However, the very-high-NaCl load of 28·6 g did result in a significant depression in food intake in the first and second 10 min periods compared with controls. Intake from 0 to 20 min was only depressed by the 28·6 g NaCl treatment relative to controls. Both the low- and high-NaCl treatments inhibited 0–30 and 0–60 min food intakes.

There was a highly significant treatment effect on water consumption (P = 0·0001) from 60 to 90 min. Both NaCl loads and 196 g PEG-200 resulted in significantly greater water consumption than controls, the value for 28·6 g NaCl being greater than those for 14·3 g NaCl and 196 g PEG. These increases in water consumption were not accompanied by food intake responses as found in the rumen studies. Increased water consumption due to osmotic loading is not, therefore, always associated with the ingestion of food.

The control abomasal tonicity values were 277 (SE 6), 279 (SE 6) and 279 (SE 6) mosmol/kg for 16 min before and 9 and 14 min after dosing respectively. Corresponding tonicity values for the 14·3 g NaCl treatment were 291 (SE 7), 606 (SE 29) and 588 (SE 27) mosmol/kg whereas for the 28·6 g NaCl treatment they were 273 (SE 5), 1012 (SE 61) and 900 (SE 67) mosmol/kg.

The increases in plasma osmolality were 0·1 (SE 0·4), 3·9 (SE 0·7) and 7·0 (SE 0·5) mosmol/kg after 10 min of feeding for the control, 14·3 g and 28·6 g NaCl treatments respectively (Fig. 6). Corresponding changes from 60 to 90 min were 1·7 (SE 0·6), −2·2 (SE 0·6) and 2·3 (SE 1·3) mosmol/kg. Plasma tonicity at 60 min was already 15 (SE 1·4) mosmol/kg higher than pre-injection values for the 28·6 g NaCl treatment, yet the sheep consumed as much food as controls from 60 to 90 min.
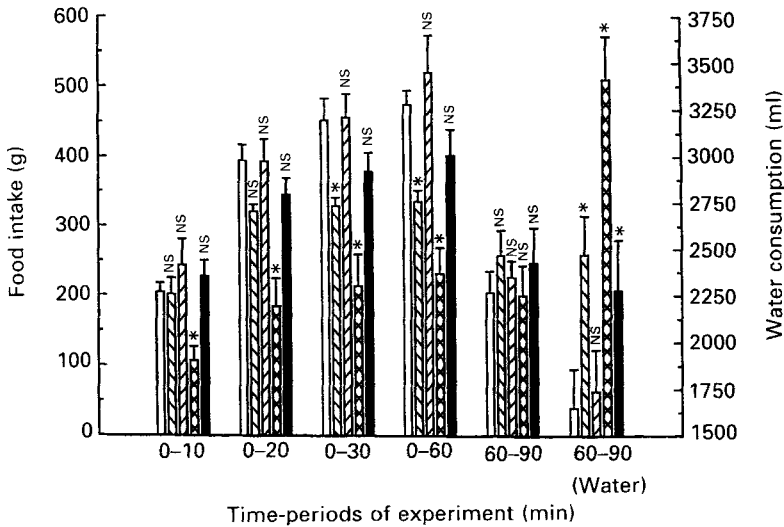
Fig. 5. Expt 5. Effect on food intake and water consumption of injecting 1·65 (control; ☐), 14·3 (▧) or 28·6 (⊠) g sodium chloride, or 98 (▨) or 196 (■) g polyethylene glycol (molecular weight 200; PEG) into the abomasum of sheep that were deprived of food for 5·5 h (n 5). NaCl and PEG were injected immediately before feeding. Intake is displayed for the time periods 0–10, 0–20, 0–30, 0–60 min when water was withheld and from 60 to 90 min when water was available. Values are means with their standard errors represented by vertical bars. Treatment means were significantly different from those for the controls for each time period using the Newman-Keuls test: *P < 0·05. NS, not significant.

Expt 6. NaCl loading of the reticulo-rumen and abomasum

From 0 to 10 min, the 50 g NaCl-rumen treatment resulted in a significantly lower intake than the controls (Fig. 7) and this result applied to the cumulative intakes up to 20, 30 and 60 min. Intake from 60 to 90 min for the 50 g NaCl-rumen treatment was significantly greater than for all other treatments. This intake response was associated with a significantly greater water consumption than for the control rumen treatment. Whilst water consumption for the 25 g NaCl-rumen treatment and the 14·3 g NaCl-abomasum treatment were also significantly greater than that for controls, the food intake responses were not different.

Despite similar increases in plasma osmolality from 0 to 10 min for the 50 g NaCl-rumen and 7·1 g NaCl-abomasum treatments (2·8 (SE 1·16) and 2·7 (SE 1·19) mosmol/kg respectively), intake was inhibited with the rumen treatment but not with the abomasal treatment. Similarly, over the first 20 min period, the plasma tonicity increased by 7·7 (SE 1·17) and 7·8 (SE 1·2) mosmol/kg for the 14·3 g NaCl-abomasum and 50 g NaCl-rumen treatments respectively, yet only the high-NaCl-rumen treatment resulted in a significant intake depression. The relationship between intake over the first 10 min and the change in rumen osmolality was highly significant (P = 0·005) with an R^2 of 0·43 and is described by equation 4:

$$y = 336·2 - 1·14x, \qquad (4)$$

where y is food intake (g) and x is the change in rumen fluid tonicity in mosmol/kg from 0 to 10 min.

The plasma tonicity for the 50 g NaCl-rumen treatment in Expt 6(b) increased to 311·2 (SE 0·9) mosmol/kg at 60 min and rose slightly to 311·6 (SE 1·5) mosmol/kg at 90 min. Despite this, a significant compensatory intake response was observed. Water consumption during this period was significantly greater for the 50 g NaCl-rumen treatment than for
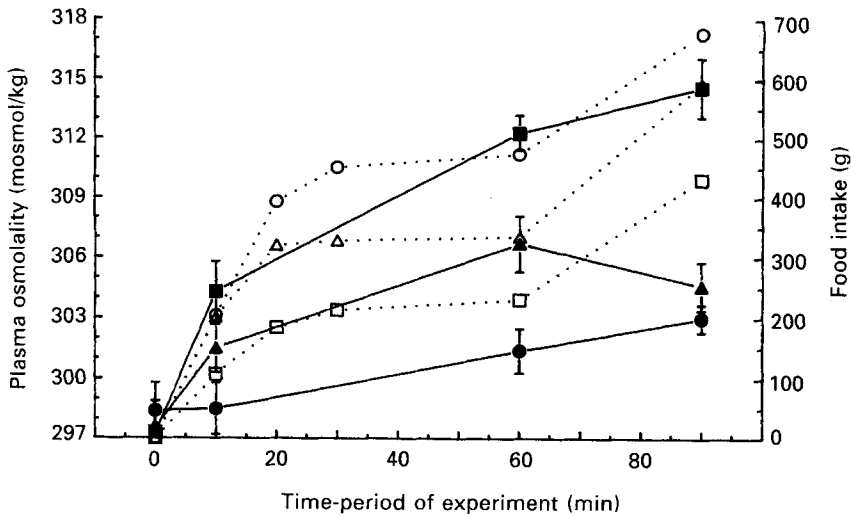
Fig. 6. Expt 5. Effect on plasma osmolality and food intake of injecting 1·65 (control; ○, ●), 14·3 (△, ▲) or 28·6 (□, ■) g sodium chloride into the abomasum immediately before feeding sheep (time 0) after a 5·5 h deprivation period. Water was withheld from 0 to 60 min but was available from 60 to 90 min. (○, △, □), Cumulative food intake; (●, ▲, ■), osmolality of plasma (*n* 5).
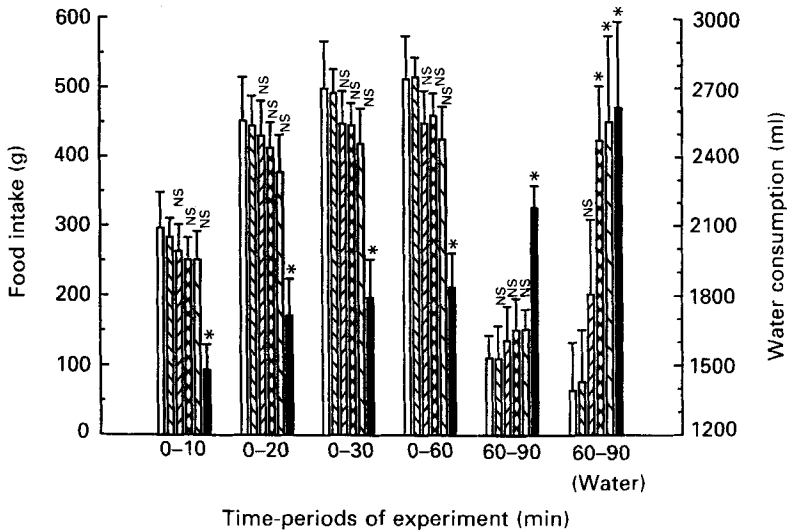

Fig. 7. Expt 6(a). Effect on food intake and water consumption of injecting 0·41 (control; □), 7·1 (▨) and 14·3 (▧) g sodium chloride into the abomasum or 1·34 (control; ▨), 25·0 (▨) and 50·0 (■) g NaCl into the reticulo-rumen of sheep that were deprived of food for 5·5 h (*n* 6). NaCl was injected immediately before feeding. Intake is displayed for the time periods 0–10, 0–20, 0–30, 0–60 min when water was withheld and from 60 to 90 min when water was available. Values are means with their standard errors represented by vertical bars. Treatment means were significantly different from those for abomasal and rumen controls for each time period using the Newman–Keuls test; *$P < 0.05$. NS, not significant.

controls, resulting in a reduction in rumen osmolality. The mean reduction was 48 (SE 15), 79 (SE 12) and 94 (SE 16) mosmol/kg for the control, 25 g and 50 g NaCl treatments respectively.

DISCUSSION

The results of these experiments indicate that the inhibition in food intake resulting from loading the rumen with NaCl or PEG-200 is caused by the increase in osmolality of rumen digesta. Increases in tonicity of abomasal digesta and blood were not responsible for the intake depression. This is the first detailed investigation of the post-ingestive mechanism explaining how NaCl depresses food intake. In order to elucidate the mechanism, attention was paid to the first 10 min of eating immediately following NaCl loading. This allowed little time for tonicity changes to occur beyond the rumen (due to solute absorption, movement of extracellular fluid into the rumen and onward passage of NaCl). Attention was also paid to the 60–90 min period during which water and food were offered and compensatory intakes were observed.

The linear reduction in food intake with increasing rumen NaCl load (Expt 1) is in agreement with previous work (Ternouth & Beattie, 1971; Phillip et al. 1981). However, the response in the present study was obtained in the first 10 min of eating compared with 60 and 30 min, being the first measurements of intake respectively in the studies mentioned above. Furthermore, the time for absorption of ions and onward passage of NaCl were 60 and 210 min in those studies respectively, compared with 10 min in the present study. These authors concluded that increasing osmolality of rumen fluid depressed food intake but they had insufficient information to arrive at that conclusion unequivocally. The food intake depression was linear in Expt 1 reported here when the NaCl load was increased up to 50 g, suggesting that small additions of NaCl would still result in some reduction in intake.

Equivalent osmotic loads (i.e. 50 g NaCl and 342 g PEG-200) into the rumen resulted in a similar food intake depression. The significant intake reduction in the first 10 min after loading with PEG indicates that the response was not related to the ionic nature of the solute but rather to its osmotic effect. This finding is contrary to that of Kato et al. (1979) who concluded that the depression in food intake was related to the concentration of sodium or potassium ions, or both, in rumen fluid rather than to the change in osmolality. However, their infusions of NaCl, KCl or PEG-300 into the rumen were started at the time food was offered and continued throughout the 2 h feeding period. Since their sheep were trained to consume their daily food allocation in a 2 h period, the rate of eating would have been high initially. By commencing the infusion at the start of the meal, probably no increase in the tonicity of rumen fluid of any consequence would have occurred until after the major portion of the meal was consumed. The osmolality values and ion concentrations reported were also determined from rumen samples taken at the end of the 2 h period. Hence, any relationships between tonicity or ionic effects and food intake are questionable. Ternouth & Beattie (1971) found the reduction in lucerne chaff intake expressed as g chaff/osmol, to be similar for NaCl, KCl, sodium acetate, sodium propionate and sodium butyrate, suggesting that neither the energy content of the solutions nor the nature of the solution was involved in the intake response. Similar reductions in intake with extracts of fresh and ensiled maize and NaCl solutions were found by Phillip et al. (1981) who concluded that osmolality of rumen fluid was the main factor responsible for the low intakes of silages. Citing the work of Kato et al. (1979) and de Jong et al. (1981) with reference to Na^+ and K^+ concentrations, it was suggested by Gill et al. (1987) that specific silage constituents rather than osmolality inhibited intakes of silages. This conclusion appears to be inappropriate because, (a) silages were not used in either of the cited studies, (b) de Jong et al. (1981) did not measure rumen concentrations of Na^+ or K^+ and (c) the

experimental protocol of Kato *et al.* (1979) has been criticized previously. Whilst silage was not used in the current experiments either, the results indicate clearly that an increase in tonicity of rumen fluid will depress intake.

The possibility that the food intake response was mediated by the abomasum following passage of NaCl or PEG from the rumen was ruled out. The osmolality of abomasal contents was elevated to 606 (SE 29) mosmol/kg by 14·3 g NaCl with no effect on food intake from 0 to 10 min. Whilst 28·6 g NaCl raised abomasal tonicity to 1012 (SE 61) mosmol/kg and did depress intake in the first 10 min, this tonicity is well beyond the physiological range. The mechanism operating to explain these unphysiological findings is unknown.

Whilst hepatic sensing of osmotic pressure has been demonstrated in the guinea-pig (Niijima, 1969) and the rat (Adachi *et al.* 1976), the existence of a true osmoreceptor is in question (Lautt, 1980). The afferent discharge rate in the hepatic nerve of the rabbit changed with altered Na^+ concentration or oncotic pressure (due to plasma proteins) of the perfusing medium, but not osmotic pressure (Andrews & Orbach, 1974, 1975). There are no reports of ion receptors or osmoreceptors in the ruminant liver. The increase in tonicity of portal and jugular plasma was shown to be almost identical during feeding in sheep after a 15 h fast (Carr & Titchen, 1978). However, loading the rumen with NaCl before eating, as in the present experiments, is likely to raise the tonicity of portal blood to a greater extent than systemic blood. There was, however, no consistent relationship between changes in the tonicity of jugular plasma and food intake in the experiments reported. This implies that the liver or any other site (e.g. brain) beyond the reticulo-rumen was not involved in sensing osmolality to depress food intake. Thirst induced by vascular changes in tonicity appears not to be involved with the intake depression either, as similar elevations in plasma tonicity following rumen and abomasal NaCl loading (up to 7·8 mosmol/kg) were not consistently associated with an inhibition of intake.

The combined results from the six experiments now reported indicate that a change in osmolality of rumen fluid is sensed in the reticulo-rumen to depress food intake. The decrease in food intake was linear with increasing NaCl load into the rumen. Although the high-NaCl loads induced large changes in rumen fluid osmolality, they are likely to be of physiological significance since rumen fluid tonicity has been shown to approach 500 mosmol/kg after feeding (Warner & Stacy, 1968). The omasum was not considered to play a part in the intake depression from NaCl loading but it cannot be eliminated by these studies. The rapid onset of the intake inhibition suggests that tonicity is being sensed in the wall of the reticulo-rumen. Epithelial receptors have been located in the reticulum, cranial sac, around the cranial and longitudinal pillars and occasionally in other parts of the rumen (Harding & Leek, 1972; Leek, 1972), but they had to be exposed to 1700 mosmol/kg from NaCl before a convincing proportion of receptors responded. It appears, therefore, that another population of receptors may be sensing tonicity within the physiological range in the rumen to mediate the observed intake depression.

The effect of NaCl loading the rumen on motility was carefully examined because excitation of epithelial receptors in the reticulo-rumen reflexly inhibited primary contractions (Harding & Leek, 1973) and an inhibition of motility could depress food intake. This possible mechanism was ruled out in the preliminary experiment in which no significant effect on frequency or amplitude of reticular contractions was recorded after raising the osmolality of the reticulum and the rumen. This confirmed the observations made by Phillip *et al.* (1981).

The addition of 4 or 8 g lidocaine to the rumen immediately before the NaCl loads did not block the inhibitory effect of NaCl on intake. This does not imply that the receptors are not in the reticulo-rumen as the concentration of anaesthetic in the region of the

receptors may not have been sufficient to block their activity. The problems with this approach include dilution of the anaesthetic in rumen contents and its breakdown by the rumen micro-organisms. Bergen (1972) demonstrated a reversal of intake inhibition from NaCl in the rumen with only 20 mg carbocaine in each of two sheep. In light of the current results and considering that only two sheep were used, the efficacy of local anaesthetics in this work is somewhat questionable.

The ability of NaCl to limit intakes of protein supplements and concentrates is likely to be due to its post-ingestive effects rather than to palatability (Grovum & Chapman, 1982). The present studies localized the receptive mechanism to the wall of the reticulo-rumen. Experiments are, therefore, needed to locate and characterize these receptors because they appear to be capable of sensing osmolality within the physiological range of rumen fluid and to depress food intake.

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